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Preliminary SAR studies on non-apamin-displacing 4-(aminomethylaryl)pyrrazolopyrimidine K_{Ca} channel blockers

Robert G. Gentles *, Shuanghua Hu, Yazhong Huang, Katherine Grant-Young, Michael A. Poss, Charles Andres †, Tracey Fiedler, Ronald Knox, Nicholas Lodge, C. David Weaver ‡, David G. Harden

Research and Development, Bristol Myers Squibb Co., 5 Research Parkway, Wallingford, CT, USA

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ABSTRACT

An exploratory SAR study on a series of potent, non-apamin-displacing 4-(aminomethylaryl)pyrazolopyrimidine K_{Ca} channel blockers is described and their selectivity against K_{Ca} channel subtypes is reported. The most potent analog, 5-chloro-*N*-(thiophen-2-ylmethyl)pyrazolo[1,5-*a*]pyrimidin-7-amine (**24**) displayed sub-micromolar activity in both a thallium flux and whole-cell electrophysiology assay and did not displace apamin in a competitive binding study.

Small conductance calcium-activated potassium (K_{Ca}) channels are voltage-insensitive and open in response to elevated levels of intra-cellular calcium.[1] These ions mediate their effect by binding to calmodulin, a protein that is constitutively associated with the channel and that undergoes a structural change induced by higher calcium occupancy.[2–6] This alteration in the protein's conformation results in opening of the channel and an associated enflux of potassium ions, and it is this current that is responsible for the afterhyperpolarization observed following the initial depolarization/repolarization phases of an action potential in neurons expressing K_{Ca} channels.[7,8]

Such afterhyperpolarizations impact neuronal excitability and can significantly modulate neuronal firing patterns.[9,10]

Three isoforms of K_{Ca} channels have been identified: K_{Ca}2.1, K_{Ca}2.2, and K_{Ca}2.3, and their distributions in both rat and human brain have been determined.[1,9,11,12] From these studies, it is apparent there exists the possibility for regional modulation of neuronal excitability, and significant efforts have been directed at identifying both pan- and sub-type-selective K_{Ca} channel blockers. Compounds with appropriate profiles may have utility in the treatment of diseases in which modulated neuronal firing would be expected to be adventitious.[13] These would include conditions such as epilepsy, depression, Parkinson's disease,[14] and schizophrenia.[15] K_{Ca} blockers may also prove helpful in the treatment of certain cognitive disorders.[16,17]

To date, a limited number of non-peptidic compounds have been reported to be active at K_{Ca} channels,[18–20] with the majority of these being thought to function by obstructing the pore of the channel. Examples include the quinoline-appended diazepines;[21] isoquinoline analogs related to bicuculline, including *N*-methyl laudanosine;[22–26] and a class of 2-aminothiazoles,[27] as shown in Figure 1. However, in a recent publication, it was reported that the 2-aminobenzimidazole, NS8593 (IC_{50} = ~500 nM) is a non-apamin-displacing K_{Ca} channel blocker that exerts its effect at a site remote from the pore region.[28] In this letter, we report a preliminary SAR study on an additional series of 5-chloro-*N*-(methylaryll)pyrazolo[1,5-c]pyrimidin-7-amines that we demonstrate are also non-apamin-displacing K_{Ca} channel blockers with activities similar to that reported for the 2-aminobenzothiazoles. In addition, we report on the K_{Ca} channel selectivities of the more potent members of this class, and describe the behaviors of the compounds in whole cell electrophysiology and apamin-displacing studies.

As discussed in a previous letter,[27] our search for novel K_{Ca} channel blockers began with an HTS screen that employed a thallium flux assay in which compounds were tested against a recombinant HEK 293 cell line-expressing specific K_{Ca} channel isoforms. This assay has been described previously,[32] and is one in which the IC_{50} obtained for the known K_{Ca} channel blocker apamin has been repeatedly demonstrated to be consistent with reported literature values. This screen resulted in the identification of a number of hits, one of the more interesting being, *N*-benzyl-5-chloropyrazolo [1,5-*c*]pyrimidin-7-amine (**1**), as shown in Figure 2.

* Corresponding author.
E-mail address: robert.gentles@bms.com (R.G. Gentles).
† Oblon Spivak, 1940 Duke Street, Alexandria, VA, USA.
‡ Vanderbilt Institute of Chemical Biology and Department of Pharmacology, Nashville, TN, USA.

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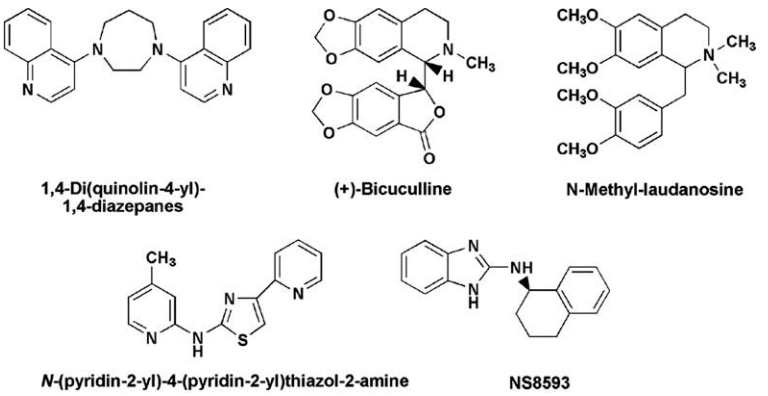

Figure 1. Previously reported small molecule blockers of K_{Ca} channels.

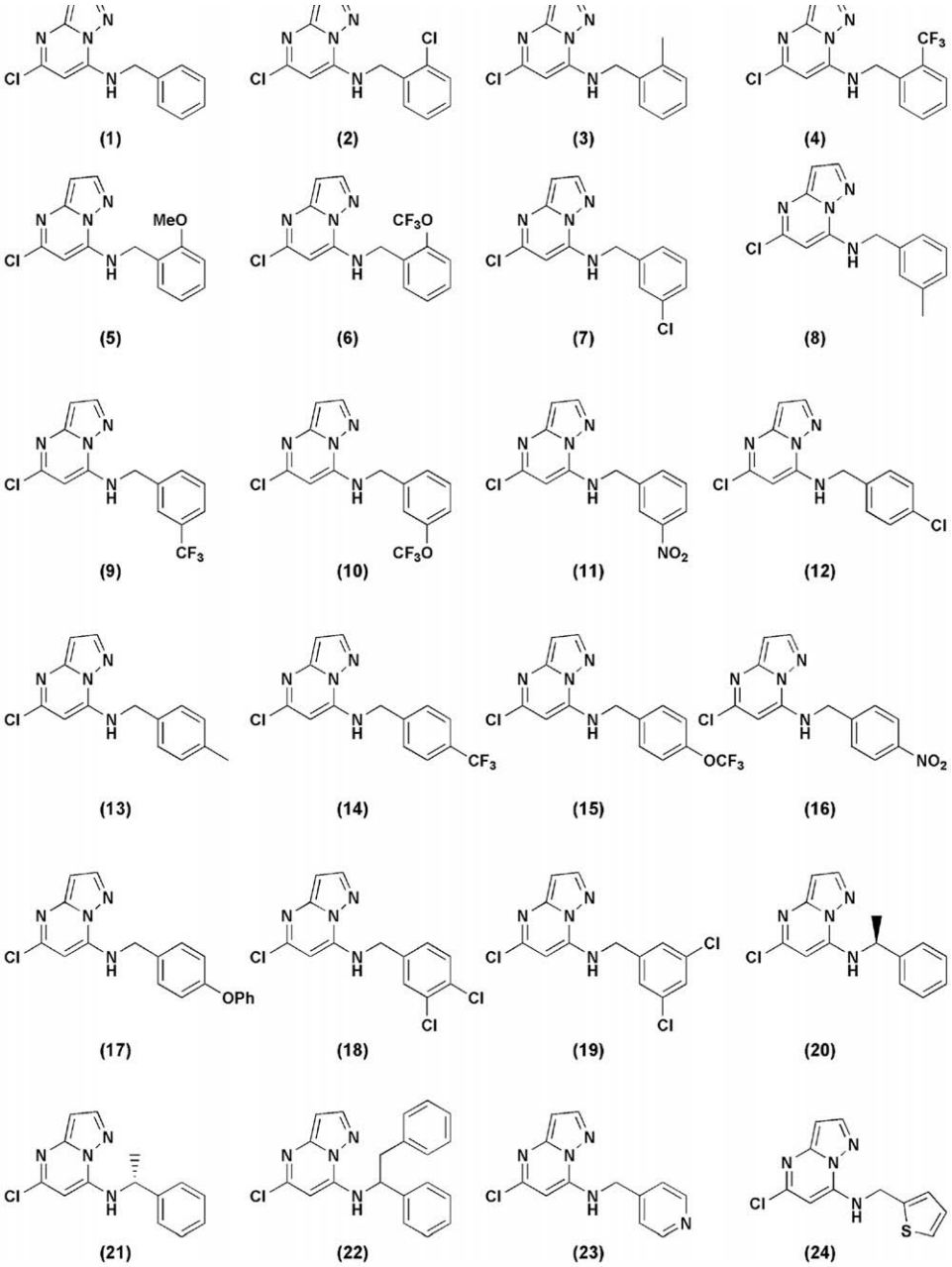

Figure 2. *N*-Methylaryl-5-chloropyrazolo[1,5-*c*]pyrimidin-7-amines evaluated for activity at K_{Ca} channels.

After confirming the structure and purity of the screening sample of **1,** the compound's IC_{50} was determined using the thallium flux and electrophysiology assays, and both values were found to be in good agreement, as can be seen in Table 1.

Given these results, we sought to develop an initial SAR around **1** to determine if this chemotype could be progressed as a series of effective K_{Ca} channel blockers. To that end, we chose to first explore the introduction of substituents at the methylene and aryl moieties of the benzylamine functionality in **1**, as shown by the analogs depicted in Figure 2.

These compounds were prepared from commercial starting materials employing standard methodology[29–31] that was modified slightly to better accommodate our specific array purification protocols, as shown in Figure 3.

The process adopted involved heating solutions of 5,7-dichloropyrazolo[1,5-*a*]pyrimidine in acetonitrile with one of a series of benzyl- or heteroarylmethyl amines overnight at 80 °C. On cooling, the desired products were isolated using preparative reverse-phase HPLC.

The compounds so obtained were then screened in the thallium flux assay, and the data associated with these experiments are presented in Table 1.

From these results it is apparent that attempts to introduce functionality at the *ortho*-position of the phenyl group resulted in significant loss of activity. This seemed unrelated to the electronic character of the substituent; electron withdrawing groups such as those in analogs, **2**, **4**, and **6**, resulted in approximately a 10- to 30-fold loss in potency, as did electron-donating substituents as seen in examples **3** and **5**.

The *meta*-position of the aryl group in **1** was more tolerant of a range of substitution. The chloro analog **7**, and the nitro derivative **11,** displayed modest losses of potency relative to the *ortho*-substituted derivatives. The methyl **8**, trifluoromethyl **9**, and trifluoromethoxy **10** derivatives were essentially equipotent, and again confirmed that the electronic character of the aromatic ring did not appear to be a key determinant of potency. Interestingly, the 3,5-dichloro analog **19** lost significant activity, in contrast to the mono-substituted analog **7**.

Introduction of functionality at the *para*-position of the pendant phenyl group was not tolerated, as can be seen with analogs, **12**–**17**. All of these analogs show a significant loss of activity and this would appear to be attributable to steric factors, as the electronic character of the pendant moieties in these compounds is quite diverse. Consistent with this observation is the inactivity of the disubstituted analog, **18**.

We next turned our attention to the substitution of the methylene element of the benzyl group in **1**. The introduction of a methyl moiety was explored in the two enantiomers, **20** and **21.** These compounds have the absolute stereochemistries (*S*) and (*R*), respectively. Interestingly, it was found that **20** was inactive at the $K_{Ca}2.3$ channel, but the (*R*)-isomer **21** was essentially equipotent with the unsubstituted derivative **1**. The immediate higher homologs in this series were not examined, but a similar dependence on appropriate stereochemistry might reasonably be expected.

In an attempt to probe the steric constraints that might exist for substituents at this position, we introduced the sterically more demanding benzyl moiety, as seen in analog **24.** This resulted in a significant loss of activity relative to the original lead **1,** which may be interpreted as giving an indication of the steric limitations of groups that can be introduced at this position. However, the potential for further optimization of this series at this position is apparent.

Lastly, we directed our attention to the use of heterocyclic replacements of the phenyl group in **1**. An initial investigation using a series of pyridinyl analogs was unsuccessful. The results observed with compound **23** were consistent with those observed with the related pyridin-3-yl and pyridin-2-yl analogs (data not shown), in that none showed any significant activity.

More encouraging, however, was the result obtained with the standard phenyl isostere, thiophene. The compound, 5-chloro-*N*-(thiophen-2-ylmethyl)pyrazolo [1,5-*c*]pyrimidin-7-amine (**24)** displayed significantly improved potency (IC_{50} = 390 nM) relative to other analogs tested in our thallium assay, and this result was supported in a related electrophysiology assay (IC_{50} = 580 nM).

In order to better understand the mechanism of the observed channel block, we examined the activity of **24** in a Scintillation Proximity Assay (SPA) to assess its ability to displace radio-labeled [^{125}I]-apamin from the $K_{Ca}2.3$ channel.[33,34] In this assay, **(24)** did not compete with the labeled ligand, a finding that suggested that this compound interacts with the channel at a site remote from that occupied by apamin. Correspondingly, we were interested to explore the possibility that this alternative binding site could be exploited to identify small molecules that would display selectivity between the different K_{Ca} channel subtypes.

Subsequently, compounds **1** and **24** were tested in the thallium flux assay against cell lines recombinantly expressing the $K_{Ca}2.1$ and $K_{Ca}2.2$ channel isoforms. As can be seen from the data in Table 2, these compounds displayed no significant selectivity between the various channel subtypes. This was similar to our previous observations with a series of apamin-displacing aminothiazole blockers.[27] Obviously, however, further work remains to be done to fully investigate the potential of this series to identify K_{Ca} channel subtype-selective blockers.

In conclusion, we present a series of 5-chloro-*N*-(arylmethyl)pyrazolo[1,5-*c*]pyrimidin-7-amines that are effective $K_{Ca}2$ blockers and appear to act remotely from the classic apamin-binding site. Specific analogs disclosed are equipotent with the only other previously reported, non-apamin-displacing K_{Ca} channel blocker, and an exploratory SAR has been developed,

Table 1
Activities of compounds **1**–**24** at the $K_{Ca}2.3$ channel in the thallium flux and electrophysiology assays

Compound	$K_{Ca}2.3$ Thallium flux % inhibition at 30 μM	$K_{Ca}2.3$ Thallium flux IC_{50}[a] (μM)	$K_{Ca}2.3$ EP IC_{50}[b] (μM)
1	113	1.1	1.60 (±0.22)
2	62	19.1	
3	75	14.6	
4	50	>30	
5	74	15.9	
6	87	10.0	
7	113	5.2	
8	91	10.7	
9	80	14.6	
10	71	11.0	
11	96	7.9	
12	30	>30	
13	46	>30	
14	43	>30	
15	62	15.4	
16	25	>30	
17	23	>30	
18	25	>30	
19	43	>30	
20		IA	
21		1.0	
22		7.5	
23		IA	
24	105	0.35	0.58 (±0.05)
	Apamin	0.000168 (±0.000136)	0.000064 (±0.000006)

[a] See Note 35.
[b] Values are means of three experiments, standard deviations are given in parentheses. IA, inactive.

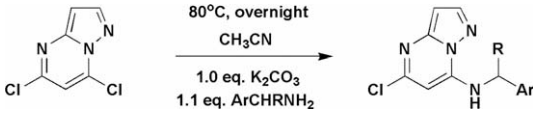

Figure 3. Synthetic method used for the synthesis of the 4-(aminomethylaryl)pyrazolopyrimidines shown in Figure 2.

Table 2
Selectivity of compounds **1** and **24** against K_{Ca} channel isoforms, K_{Ca}2.1, K_{Ca}2.2, and K_{Ca}3.1

Compound	K_{Ca}2.1 Thallium flux IC_{50}[a] (μM)	K_{Ca}2.2 Thallium flux IC_{50}[a] (μM)	K_{Ca}2.3 Thallium flux IC_{50}[a] (μM)
1	1.19	1.10	1.12
24	0.30	0.53	0.35

[a] See Note 35.

and vectors for the further optimization of this series have been identified.

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35. Each concentration of a given sample was tested in triplicate. The mean percent inhibitions of the triplicates for each concentration were used to determine an IC_{50} value based on a single-site logistic fit (Microsoft XLFit). For selected compounds, IC_{50} determination were repeated on three separate occasions and the mean and the standard deviation is reported. Overall, the variability of IC_{50} determination was observed to be within one 1/2 log unit as seen with the standard deviations reported.